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AN AUTORADIOGRAPHIC DEMONSTRATION OF STOMACH TOOTH RENEWAL IN *PHYLLAPLYSIA TAYLORI* DALL, 1900 (GASTROPODA: OPISTHOBRANCHIA)

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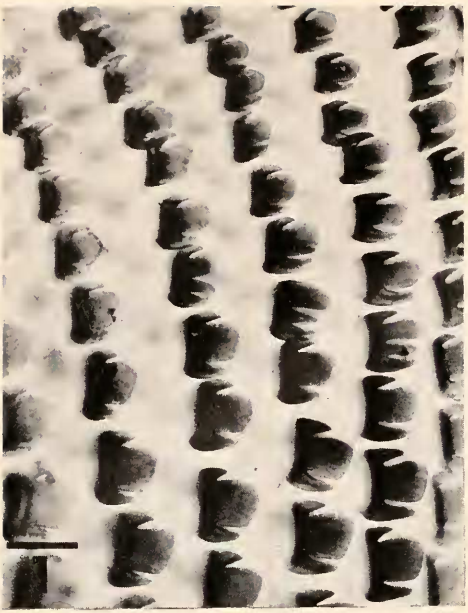
Phyllaplysia taylori Dall, 1900, a small, green aplysid (Order Anaspidea, Family Aplysiidae—the "sea hares") common on *Zostera marina* plants along northeastern Pacific shores, has recently been shown to have a diet consisting largely of sessile diatoms (Beeman, 1968). The jaws, radula, and stomach teeth of *P. taylori* are especially well adapted to handling this siliceous, abrasive material. The broad, scraping radula (Fig. 1) removes the food from the substrate and draws it inward between vertical jaws composed of numerous, tightly packed, tiny, hard rods. The esophagus then conveys the food to the triturating stomach or "gizzard," a broad muscular sac (illustrated in McCauley, 1960) lined with corneous intermeshing teeth (Fig. 2). The triturating action here can be so efficient that the identity of food particles in aplysids may not be discernible beyond this point.

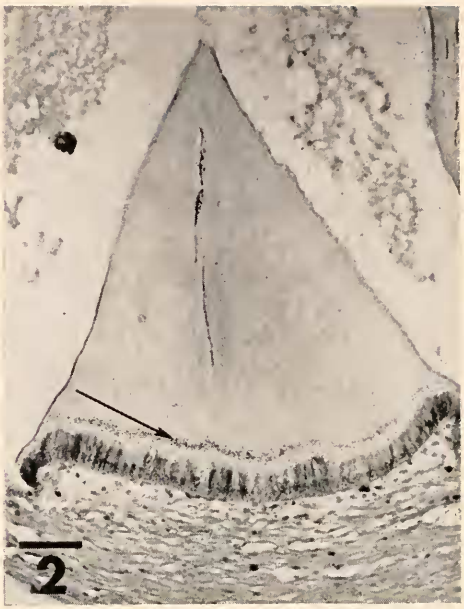
Surfaces involved in the grinding of abrasive foods must be repaired or replaced. The rate of replacement for radular teeth, which are replaced as discrete units through slow growth of the radular strap from its posterior origin, has been reported for two pulmonates (Runham, 1963), but nothing is known of such replacement rates in opisthobranchs. The stomach teeth likewise show wear and nothing has been reported of their replacement mechanism or rate. Here my studies using H^3-thymidine autoradiography, although designed primarily to study reproductive mechanisms (Beeman, 1966), have provided pertinent information.

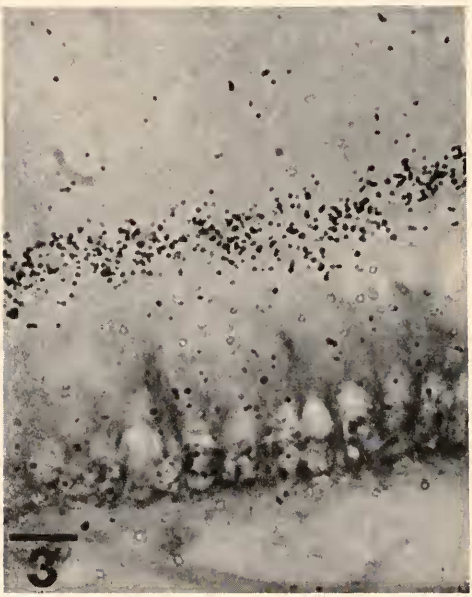
Methods and Materials

Specimens of *Phyllaplysia taylori* for this experiment were taken from Elkhorn Slough, California and maintained in special cages in large outdoor seawater tanks (*ca.* 14–16 C) at Hopkins Marine Station. The thymidine used was tritiated at the 5-methyl position and had a specific activity of 2000 mc/mM as supplied by the New England Nuclear Corporation, Boston, Massachusetts.

The experimental series for this study was started on July 25, 1964. The animals used were typically about 7×22 mm (*ca.* 190 mg live weight), but they







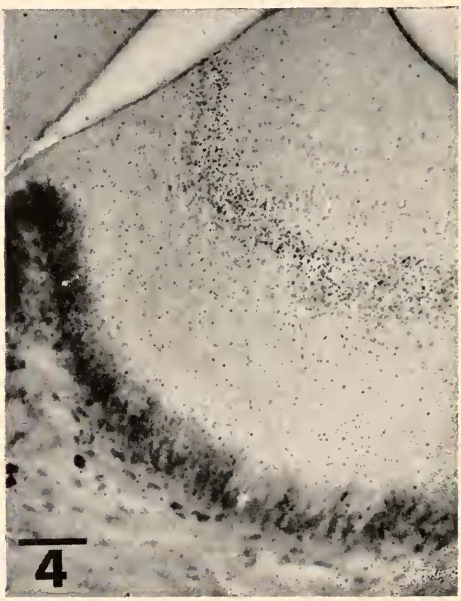


FIGURE 1. Lateral radular teeth of *Phyllaplysia taylori*. Acetocarmine. Scale line represents 50 μ.

FIGURE 2. An autoradiogram showing a sagittal section of a triturating stomach tooth from a *Phyllaplysia taylori* killed 24 hours after injection of tritiated thymidine. Arrow indicates a line of silver grains over the labeled area. Mayer's haemalum. Scale line represents 50 μ.

FIGURE 3. Basal detail of the tooth shown in Figure 2. Focus is in the plane of the silver grains. Scale line represents 10 μ.

ranged from 4 × 18 mm (70 mg) to 9 × 28 mm (536 mg). Experimental individuals were injected with a 31 gage needle into the left anterior quadrant of the hemocoel with 5 μc of H^3-thymidine per gram of body weight and returned to running seawater until sacrificed. Two or three animals were killed by rapid injection of Bouin's seawater solution at each post-injection interval of 1, 2, 4, 10, and 48 hours and 7, 10, 14, 20, 23, and 30 days. Whole fixed specimens, or dissected viscera, were embedded in paraffin, cut at 7 μ, and processed on slides via regular microtechnique. These slides were dipped in Ilford Nuclear Research Emulsion Type K5, exposed in darkness at 5 C for 33, 34, or 78 days, and then developed (six minutes in undiluted Kodak D-19 developer), fixed, rinsed, stained with Kessel's modification of Mayer's haemalum stain for 2 to 15 minutes, "blued" in running tap water for 15–20 minutes, run through an alcohol series, cleared in toluene, and mounted in Permount resin (Joftes, 1963; Holland and Giese, 1965).

Results

Histological preparations which include sagittal sections of the stomach teeth of animals injected with H^3-thymidine show that the teeth have taken up the radioactive label (Figs. 2–4). One hour after injection no label was found associated with the stomach teeth or with their basal cells. Within 10 hours a distinctly labeled band is present at the base of each tooth, just above, but not in the basal cells. Within 24 hours this label line has moved up the tooth, clear of the base, and by 20 days it has almost reached the grinding tip. Total replacement occurs in about 25 days, as no label is present after that post-injection interval. There is no autoradiographic evidence of the migration of labeled nuclei into the growing tooth; the label was found in the non-cellular, translucent matrix of the tooth. Measurement of the position of the labeled band in a total of 17 teeth in five specimens indicates an average growth rate of about 4.9 μ per day. This is a daily replacement of about 4.2% of the mean total height (117.4 μ) of the teeth.

Discussion

The triturating stomach teeth of *P. taylori* are obviously being renewed by secretion of an extracellular matrix from their basal cells. This contrasts with the results of Holland (1965) who autoradiographically demonstrated the incorporation of labeled nuclei into the growing teeth of the sea urchin. There is no evidence of stomach teeth being replaced as discrete units as are radular teeth, but it is conceivable that this could occur on an irregular basis following accidental removal of a secreted tooth.

The main material being secreted into the stomach teeth is probably chitin; the jaws, radula, and stomach teeth of aplysids are usually referred to as chitinous. The positive chitosan color test and the chromatographic demonstration of N-acetylglucosamine, the basic saccharide of chitin, by Winkler (1960) suggest that chitin is present in the stomach teeth of *Aplysia californica* Cooper, 1863, a related aplysid with very similar digestive structures.

Figure 4. An autoradiogram of a triturating stomach tooth from a *Phyllaplysia taylori* killed seven days after injection of tritiated thymidine. Mayer's haemalum. Scale line indicates 30 μ.

The nature of the labeling is not clear. The purpose of H^3-thymidine injections is to label newly synthesized DNA. It has been well established (see review by Holland, 1964) that administration of H^3-thymidine causes nuclei to achieve a specific and stable label. However, labeled nuclei are not migrating into the renewed matrix of the stomach tooth. Most firm animal structures are composed of protein or a mucopolysaccharide framework stiffened by additional protein cross-linkages or mineral deposition (Brimacombe and Webber, 1964). The stomach teeth, which at least almost certainly contain chitin, fit this pattern. It is possible that the H^3-thymidine is involved in the formation of the mucopolysaccharide framework. Thymidine diphosphate mannose in some plants and uridine diphosphate glucose, *etc.* in animals are known precursors of mucopolysaccharide. Thymidine might get into the latter compounds as the nucleoside specificity evidently is not as great in saccharide syntheses as in nucleic acid syntheses (Leloir, 1964). However, due to the low stability of the nucleoside bondings to these precursors, the nucleosides would almost certainly stay behind when the synthesized sugars are released from the cell. This would not account for the situation in the stomach teeth, where little or no label is seen to remain in the secreting cells.

There are other ways in which one might account for the presence of H^3-thymidine, or its breakdown products, in the stomach teeth. Holland (1964) has illustrated the steps, in mammals, by which thymidine is degraded to beta-aminoisobutyric acid, which in turn is rapidly excreted in the urine. Although it seems unlikely that beta-aminoisobutyric acid would find its way into the mucopolysaccharide of the tooth, it is not known what other degradation products might occur in mollusks. As Potter (1959) has pointed out, it is not even known how some thymidine breakdown products, which occur when thymidine is incubated with mammalian liver slices, find their way into the Kreb's cycle. Tritiated water, as well as tritiated beta-aminoisobutyric acid, is produced when thymidine tritiated at the 6-hydroxyl group of the pyrimidine ring is degraded (Rubini, Cronkite, Bond and Fliedner, 1960). It is not known if tritiated water occurs when the 5-methyl tritiated thymidine, used in the present experiment, is degraded. Since the degradation products are not known, it is not clear what the non-nuclear label in the stomach teeth may represent. It may be as simple as a labeled acetyl group shunted into the mucopolysaccharide synthesis. Such tritiated acetyl groups would be expected to concentrate in areas of very significant acetylation. The stomach tooth, with its rapid replacement rate, is such a special area. The autoradiographic image resulting from the incorporation of tritiated water would probably be insignificant, due to dilution.

The recent report by Sakai and Kihara (1968) that labeled uridine was unexpectedly incorporated into mouse liver and rat kidney protein supports a suspicion that protein may be bearing the label which appears in the stomach teeth of *P. taylori* after the intrahemocoelic injection of H^3-thymidine. These workers also did not know the mechanisms by which the unexpected labeling occurred, but their work and that of the present study, suggest caution in the interpretation of experimental results, such as autoradiographic images, which follow the *in vivo* administration of labeled thymidine and uridine to higher organisms.

Tooth production and tooth destruction normally must be balanced as I have found only full-size, nicely intermeshing teeth in the functional area of the triturat-

ing stomach. Tooth growth probably normally exceeds the minimum need; the shape and size of the teeth being maintained by the milling action of their intermeshing motion. Such a mechanism would account for the addition of well-fitted teeth to the edge of the grinding area and for the enlargement of teeth in growing animals.

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Summary

1. Brief exposure to H^3-thymidine *in vivo* labels the secretion which basally renews the stomach teeth of *Phyllaplysia taylori*.

2. A line of radioactive label migrates from the base of an average stomach tooth to its tip in about 25 days. This indicates daily replacement of about 4.2% of the mean tooth height.

3. There is no autoradiographic evidence of the migration of labeled nuclei into the growing tooth; the label was found in the non-cellular, translucent matrix of the tooth.

4. The nature of the labeling is not clear. The incorporation of the label into the tooth matrix, probably mainly composed of chitin, may be as simple as the shunting of labeled acetyl groups into the mucopolysaccharide synthesis.

5. Tooth growth evidently normally exceeds the rapid wear caused by an abrasive diet of diatoms. The size and shape of the teeth is maintained by the milling action of their intermeshing motion.

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